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The inverse association of HDL-cholesterol with future risk of hypertension is not modified by its antioxidant constituent, paraoxonase-1: The PREVEND prospective cohort study

Running Title: HDL-cholesterol, PON-1, and future hypertension

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Figures [2]

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Abstract

Background: High-density lipoprotein cholesterol (HDL-C), an established risk marker for atherosclerotic cardiovascular disease (CVD), has been shown to be inversely and independently associated with incident hypertension. Paraoxonase-1 (PON-1) is an HDL-bound esterase enzyme which is associated with CVD, but its relationship with incident hypertension has not been previously investigated. We aimed to evaluate the prospective association between PON-1 and hypertension risk.

Methods: PON-1 arylesterase activity was measured in serum at baseline in 3,988 participants without pre-existing hypertension in the Prevention of Renal and Vascular End-stage Disease (PREVEND) prospective population-based study. During a median follow-up of 10.7 years, 1,206 participants developed hypertension.

Results: In age- and sex-adjusted analysis, the hazard ratio (95% CI) for incident hypertension per 1 standard deviation increase in PON-1 was 1.01 (0.96 to 1.07; p=0.656), which remained non-significant after adjustment for several established hypertension risk factors and other potential confounders (0.99, 0.93 to 1.05; p=0.764). The association was also non-existent on further adjustment for HDL-C (1.00 (0.94 to 1.06; p=0.936) and did not importantly vary across several clinical subgroups. In analyses in the same set of participants, HDL-C was continuously inversely and independently associated with hypertension risk; the association persisted after further adjustment for PON-1 activity and was not modified by PON-1 activity.

Conclusions: In this Caucasian cohort of men and women, HDL-C, but not its anti-oxidant constituent - PON-1, is inversely, continuously and independently associated with future risk of hypertension. The association is independent of and not modified by PON-1.

Keywords: paraoxonase-1; HDL cholesterol; antioxidant; risk factor; hypertension

1. Introduction

High-density lipoprotein cholesterol (HDL-C), which is included in the commonly used cardiovascular risk algorithms,[1-4] is an important risk factor for atherosclerotic cardiovascular disease (CVD).[5] A number of studies have also shown HDL-C to be inversely and independently associated with the risk of hypertension[6-9] which is the most common modifiable risk factor for CVD[10] and is a leading cause of death globally.[11] Indeed, hypertension and CVD share common antecedent risk factors (such as obesity, diabetes, and dyslipidaemia)[12] and pathophysiological pathways such as oxidative stress and inflammation.[13-15]

Paraoxonase-1 (PON-1), an HDL-bound esterase enzyme with well-established antioxidant and anti-inflammatory properties,[16-19] has been shown to be associated with CVD risk; though the evidence suggests that the association is partly dependent on HDL-C levels.[20] Since oxidative stress is known to play a central role in the pathogenesis of hypertension,[21] the underlying mechanism by which HDL may contribute to lowering the risk of incident hypertension is via its antioxidant properties. Considerable evidence suggests that the ability of the HDL to counter oxidative stress (via inhibition of oxidative modification of low-density lipoproteins) have been proposed to be largely attributable to its PON-1 component.[19, 22, 23] Given the broad body of evidence on the close relationship between HDL and PON-1, it is plausible to hypothesize that PON-1 may also be related to the risk of hypertension. Remarkably, little is known about the association between PON-1 and hypertension. Majority of published studies have been based on animal models[24] or cross-sectional data in humans[25, 26] Though low circulating PON-1 activity has been observed in patients with hypertension[27], the nature of the relationship between PON-1 and hypertension is also unclear. Whilst findings from some of these studies have suggested a protective association of PON-1 on hypertension risk,[25, 26] a causal relationship between PON-1 and blood pressure has been suggested in other reports.[24, 28] It is also unknown if higher PON-1 activity is associated with an attenuated risk of hypertension among apparently healthy populations. In this context, our primary objective was to assess the nature and magnitude of the prospective association between serum PON-1

activity and future risk of hypertension in a Caucasian population who were apparently free from hypertension and other pre-existing diseases at baseline. A secondary objective was to re-evaluate the expected inverse association of serum HDL-C concentration with risk of incident hypertension in the same set of participants, and to evaluate if the association is independent or modified by PON-1 activity.

2. Methods

The STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines for reporting observational studies in epidemiology was used during the conduct of this study (**Appendix 1**).[29]

2.1. Study Design and participants

The current analysis employed the Prevention of Renal and Vascular End-stage Disease (PREVEND) study. It is a prospective population-based investigation of albuminuria, renal and cardio-metabolic disease in a large cohort recruited from a general population setting.[30] The study was began in 1997 and involved recruitment of participants aged 28-75 years who were inhabitants of the city of Groningen in The Netherlands. The details of the study design and recruitment processes have been reported elsewhere.[31, 32] Baseline measurements were performed between 1997 and 1998 in a cohort comprising of 8,592 participants. In the current analysis, we excluded participants with hypertension, CVD, renal disease, or malignancy at baseline; which left 3,988 participants with non-missing information on PON-1 activity, HDL-C concentrations, relevant covariates, and incident hypertension. The local medical ethics committee of the University Medical Center Groningen approved the protocol for the PREVEND study and it was carried out in accordance with the Declaration of Helsinki. Written informed consent was provided by all participants.

2.2. Risk factor assessment

During two outpatient visits, baseline data were collected on demographics, physical measurements (including anthropometrics), prevalent medical conditions, and use of medications. Additional information on use of medications was collected using data from pharmacy registries of all community pharmacies in the city of Groningen. Blood pressure was assessed on the right arm whiles the subject was in the supine position; measurements were done every minute for 10 and 8 min on visit 1 and 2 respectively, using an automatic device (Dinamap XL Model 9300; Johnson-Johnson Medical, Tampa, FL). The mean blood pressure of the last two readings of both visits was recorded. Venous blood samples were taken from participants between 08:00 and 10.00 hours in the morning after an overnight fast and 15 minutes of rest. Plasma samples were centrifuged at 4 ºC. The collected serum samples were stored at -80 ºC until analysis. The enzymatic activity of serum PON-1 was measured as its arylesterase activity, i.e. as the rate of hydrolysis of phenyl acetate into phenol, as described in previous papers.[20, 33] The inter-assay CV was 8%. It has been shown that arylesterase activity measured with this assay is positively correlated PON-1 enzymatic activity toward paraoxon.[34] Total cholesterol and plasma glucose were measured by a dry chemistry method (Eastman Kodak, Rochester, New York). HDL-C was measured by a homogeneous method (direct HDL, Aeroset System; Abbott Laboratories, Abbott Park, Illinois). Standard methods were used to measure apolipoproteins (Apo) A-1 and B, high sensitivity C-reactive protein (hsCRP), serum creatinine, and serum cystatin C.[35-39] The mean of two 24-hour urine collections was used for estimating urinary albumin excretion (UAE). Urinary albumin was determined by nephelometry (BNII; Dade Behring Diagnostic, Marburg, Germany). Estimated glomerular filtration rate (eGFR), was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) combined creatinine-cystatin C equation.[40] Insulin resistance was estimated according to the homeostasis model assessment of insulin resistance (HOMA-IR) (the product of fasting glucose [mmol/l] and insulin [units/ml] divided by the constant 22.5[41]).

2.3. Ascertainment of incident hypertension

Development of hypertension during follow-up was used as the primary outcome for this study. Incident hypertension was defined as systolic blood pressure (SBP) of ≥140 mm Hg, a diastolic BP of ≥90 mm Hg, or the use of antihypertensive medication, in accordance with recommendations from the Seventh Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.[42] We also used this definition to ensure consistency with previous reports[6-9] and which would enhance comparison. Data on the use of antihypertensive medications were ascertained by administration of questionnaires at each examination and from community pharmacies in the city of Groningen, which covers complete information on drug use in 95% of PREVEND participants.

2.4. Statistical analyses

Baseline characteristics of study participants were presented overall and according to the development of hypertension during follow-up. Normally distributed variables were presented as mean (standard deviation, SD) and skewed variables as median (IQR, interquartile range). Skewed variables (such as UAE, HOMA-IR, and hsCRP) were natural logarithm ($\log_e$) transformed to achieve approximately normal distributions before the main analysis. Age- and sex-adjusted cross-sectional associations of serum PON-1 activity with risk markers for hypertension were assessed. Cox proportional hazards models were used to assess to estimate hazard ratios (HR) of serum PON-1 activity with incident hypertension risk, after confirmation of the proportionality of hazards assumptions.[43] Hazard ratios were calculated per 1 SD higher serum PON-1 values and by quartiles defined according to the baseline distribution of serum PON-1 values. To assess the independence of the association between PON-1 and hypertension risk, HRs were calculated with progressive adjustment for (i) age and sex; (ii) other established hypertension factors [smoking status, history of diabetes, SBP, total cholesterol, body mass index (BMI), parental history of hypertension, alcohol consumption, and eGFR]; (iii) other potential confounders (UAE, HOMA-IR, and hsCRP); and (iv) HDL-C. We conducted formal tests of interaction to evaluate if the association was modified by age, sex, and other risk markers for hypertension. To put our findings into context, direct

comparisons were made to associations of HDL-C with incident hypertension in the same set of participants. To assess the shape of the association between HDL-C and hypertension risk, we fitted multivariate-adjusted fractional polynomial models. To avoid double counting of the hypertension burden, studies of the global and national burden of risk factors now base their data on SBP measurements due to the strong correlation between SBP and DBP.[44] Moreover, SBP has been considered to be a better predictor of health outcomes than DBP.[45] Given this, we conducted subsidiary analyses which re-assessed the associations of PON-1 and HDL-C with risk of hypertension, as defined by SBP ≥ 140 mmHg and/or use of antihypertensive medication. To limit potential biases due to pre-existing disease, we also conducted subsidiary analysis which excluded participants with type 2 diabetes at baseline. Stata version 14 (Stata Corp, College Station, Texas) was employed in all statistical analyses.

3. Results

3.1. Baseline characteristics and correlates of paraoxonase-1 activity

Baseline clinical and laboratory characteristics of the whole cohort and comparison of individuals who developed new-onset hypertension versus individuals who remained free of hypertension are shown in **Table 1**. The mean age of overall participants at baseline was 45 (SD 11) years and the mean (SD) for PON-1 was 56.4 (18.0) U/L. Individuals who developed hypertension during follow-up were more likely to be male, older, obese, have higher BP, have diabetes, and more likely to be smokers at study entry compared with those who did not develop hypertension. Levels of total cholesterol, non-HDL-C, Apo B, glucose, hsCRP, and UAE were higher at baseline in individuals who developed hypertension compared with those who did not develop hypertension. Baseline HDL-C concentrations were lower in those who developed incident hypertension versus those who did not develop incident hypertension, but PON-1 activity was not significantly different between the groups. PON-1 was weakly correlated with waist-to-hip ratio, total cholesterol, HDL-C, Apo A-1, glucose, and hsCRP. The strongest correlations were observed with HDL-C ($r = 0.16$), Apo A-1 ($r=0.16$), and total cholesterol ($r = 0.10$). Baseline PON-1

values were significantly lower in men than in women, and were higher in current alcohol consumers compared with non-consumers (**Table 2**).

3.2. Paraoxonase-1 activity and risk of incident hypertension

During a median (IQR) follow-up of 10.7 (5.5-11.6) years, 1,206 subjects developed first-onset hypertension. In age and sex adjusted analysis, the HR for incident hypertension per 1 SD increase in PON-1 was 1.01 (95% CI, 0.96 to 1.07; p=0.656); which remained non-significant in analyses further adjusted for several established hypertension risk factors (smoking status, history of diabetes, SBP, total cholesterol, BMI, parental history of hypertension, alcohol consumption, and eGFR) and additional adjustment for $\log_e$ UAE, $\log_e$ HOMA-IR, and $\log_e$ hsCRP. This association remained consistently absent after additional adjustment for HDL-C (HR, 1.00; 95% CI, 0.94 to 1.06; p=0.936). Substituting total cholesterol and HDL-C for apolipoproteins B and A-1 respectively resulted in similar associations (data not shown). The associations were also unaltered when total cholesterol was substituted with non-HDL-C. The non-significant associations were maintained in analyses by quartiles of the baseline distribution of PON-1 values (**Table 3**). The association was not modified by any clinically relevant characteristic (**Appendix 2**). In a subsidiary analysis with incident hypertension now defined as SBP ≥ 140 mmHg and/or use of antihypertensive medication, the HRs remained similar (**Appendix 3**).

3.3. HDL-C and risk of incident hypertension

In fitting fractional polynomial models, there was a continuous and inverse association of HDL-C with risk of hypertension (**Figure 1**). An age and sex-adjusted model suggested a curvilinear shape; whiles in the multivariate-adjusted model, there was a better fit with a linear shape. Comparing the top quartile versus bottom quartile of serum HDL-C concentrations, the age and sex adjusted HR for incident hypertension was 0.62 (95% CI: 0.52 to 0.74; p<0.001), which was minimally attenuated to 0.76 (95% CI: 0.63 to 0.92; p=0.005) following further adjustment for several established risk factors. The association remained consistent on further adjustment for $\log_e$ UAE, $\log_e$ HOMA-IR, and $\log_e$ hsCRP and

was unchanged on additional adjustment for PON-1(HR, 0.80; 95% CI: 0.66 to 0.98; p=0.030) (**Table 4**). Substituting total cholesterol for Apo B or non-HDL-C yielded essentially similar associations. The associations persisted when incident hypertension was defined as SBP ≥ 140 mmHg and/or use of antihypertensive medication (**Appendix 4**). The associations also remained similar in analyses that excluded participants with type 2 diabetes at baseline (**Appendix 5**). Except for evidence of effect modification by SBP (p for interaction = 0.028), the association between HDL-C and incident hypertension was not significantly modified by other clinically relevant characteristics including PON-1. A strong inverse association was observed in subjects with lower SBP (< 119 mmHg) compared to a modest association in subjects with higher SBP (≥ 119 mmHg) (**Figure 2**).

4. Discussion

4.1. Key findings

To our knowledge, this is the first prospective study to date to examine the association of PON-1 with incident hypertension in a general population setting. In this well characterized and established cohort without histories of CVD and hypertension at baseline, we observed no association between baseline PON-1 activity and future risk of hypertension. The null association was not modified by any clinically relevant characteristic including HDL-C. In the same set of participants, we observed a continuous decrease in hypertension risk with increasing levels of HDL-C. Further large-scale studies are however required to determine whether a curvilinear or linear shape would better describe the HDL-C-hypertension relationship. The association remained independent of and was not modified by serum PON-1 activity. The association also remained persistent when incident hypertension was defined as SBP ≥ 140 mmHg and/or use of antihypertensive medication or when participants with a prevalent history of type 2 diabetes were excluded.

4.2. Comparison with previous studies

Although a number of population-based cohort studies have consistently shown serum PON-1 activity to be inversely associated with the risk of CVD; to our knowledge, the prospective association of PON-1 with the risk of incident hypertension has not been previously investigated. Findings from this large-scale prospective cohort study provide novel evidence that serum PON-1 is not associated with the future risk of hypertension. Although our findings of an independent and inverse association between HDL-C and incident hypertension are consistent with findings of previous population-based studies,[6-9] our analyses also provide relevant findings that have not been addressed in these previous studies. First, the association was independent of several confounders as well as of PON-1. Second, except for SBP, the association was not modified by PON-1 or any of the clinical relevant characteristics assessed. Finally, our subsidiary analyses in which hypertension was based on only SBP and/or use of antihypertensive medication, provided results which were similar to findings reported in the main analyses.

4.3. Possible explanations for findings

Factors such as endothelial dysfunction, inflammation and oxidative stress have been implicated in the pathophysiology of hypertension.[13-15] Lipids have been suggested to play a role in the development of hypertension, but the biological mechanisms involved in this process are not very well understood. Whereas lipids and lipoprotein parameters such as total cholesterol, low-density lipoprotein (LDL) cholesterol, non-HDL-C, triglycerides and apolipoprotein B have been shown to be associated with an increased risk of hypertension, HDL-C is protective[6-9] as demonstrated in the current analyses. It has been postulated that the main underlying mechanism between atherogenic lipid abnormalities or dyslipidemia and the development of hypertension is via endothelial dysfunction.[46] Long-term toxic exposure of the endothelium to the pro-atherogenic lipoprotein fraction may cause dysfunction of the endothelium, which results in peripheral vascular resistance, arterial stiffness, or decreased arterial compliance, and subsequently leads to elevated BP at rest.[6, 7, 47, 48] On the other hand, HDL may be protective of hypertension through its antioxidant and anti-inflammatory effects which reduce damage to the blood

vasculature.[49] HDL also has antithrombotic activities and is able to mop cholesterol present within atheromatous arteries.[50, 51] PON-1, which has well-known antioxidant and anti-inflammatory properties;[16-19] is an important component of HDL and has its site of action on HDL.[52-54] Accumulating evidence suggests that the ability of the HDL fraction to prevent LDL oxidation can be attributed to PON-1 activity. Given the consistent body of evidence on the protective association between HDL-C and cardio-metabolic outcomes, the finding of a null association between PON-1 and hypertension is unexpected. However, we have also recently shown no evidence of an association between PON-1 and type 2 diabetes and replicated the inverse independent association between HDL-C and type 2 diabetes using data from the PREVEND study.[55] The overall data suggest there may be important pathophysiologic differences between HDL-C and PON-1 in the pathogenesis of these cardio-metabolic outcomes. There is a possibility that these findings might reflect the different HDL subclasses or particle sizes, which appear to have differential protective effects on LDL oxidation.[56] Furthermore, as measurements of PON-1 in the PREVEND study involved prolonged serum storage at -80 ºC, it is also possible the null effect could be due to under-estimation of the association due to sample degradation. Indeed, PON-1 values in our sample were lower compared with previous studies of PON-1 in subjects with hypertension.[25, 26] It has however been demonstrated that PON-1 shows no loss of activity after prolonged storage in frozen samples and on repeated freeze-thawing over two years. Nevertheless, there might be an approximate loss in activity of 20% maximally over seven years.[57] There is limited evidence available on the topic and therefore further large-scale studies with measurements of these markers are warranted to confirm or refute these findings.

4.4. Strengths and limitations

Several methodological strengths of the current study deserve mention. This is the first comprehensive analyses of the observational epidemiological prospective association of PON-1 and HDL with the risk of incident hypertension in a single investigation. Participants in the PREVEND study were recruited from a general population setting and apart from exclusion of hypertensive individuals at baseline, those with

pre-existing diseases such as CVD, renal disease, or malignancy were also excluded at baseline. Analyses was comprehensive and robust which included adjustment for a comprehensive set of lifestyle and biochemical markers, assessment of any dose-response relationships, evaluation of any evidence of effect modification, and several sensitivity analyses. Limitations of the current analyses include the potential for residual confounding being an observational study, absence of data on HDL subfractions, the potential bias resulting from regression dilution because of the absence of data on repeat measurements of these markers, and inability to generalize the findings to other ethnicities.

5. Conclusion

In a general population cohort of Caucasian men and women, serum HDL-C concentration is independently and inversely associated with hypertension risk in a continuous fashion. Notably, PON-1, which is the main anti-oxidant constituent of HDL-C, is not associated with future risk of hypertension.

Conflicts of Interest

None.

Author Contributions

SKK, SJLB, RWJ and RPFD conceived and designed the study. LMK, SJLB, RWJ and RPFD acquired data. SKK analyzed and interpreted the data. SKK drafted the manuscript. SKK, LMK, SJLB, RWJ and RPFD critically revised the manuscript for important intellectual content. RPFD supervised the study. SKK is the guarantor of this work, and as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

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Author contributions

Conception and design: Setor K. Kunutsor, Stephan J.L. Bakker, Richard W. James, Robin P.F. Dullaart; Acquisition of data: Stephan J.L. Bakker, Richard W. James, Robin P.F. Dullaart; Analysis and interpretation of the data: Setor K. Kunutsor; Drafting of the article: Setor K. Kunutsor; Critical revision of the article for important intellectual content: Setor K. Kunutsor, Stephan J.L. Bakker, Richard W. James, Robin P.F. Dullaart; Final approval of the article: Setor K. Kunutsor, Stephan J.L. Bakker, Richard W. James, Robin P.F. Dullaart

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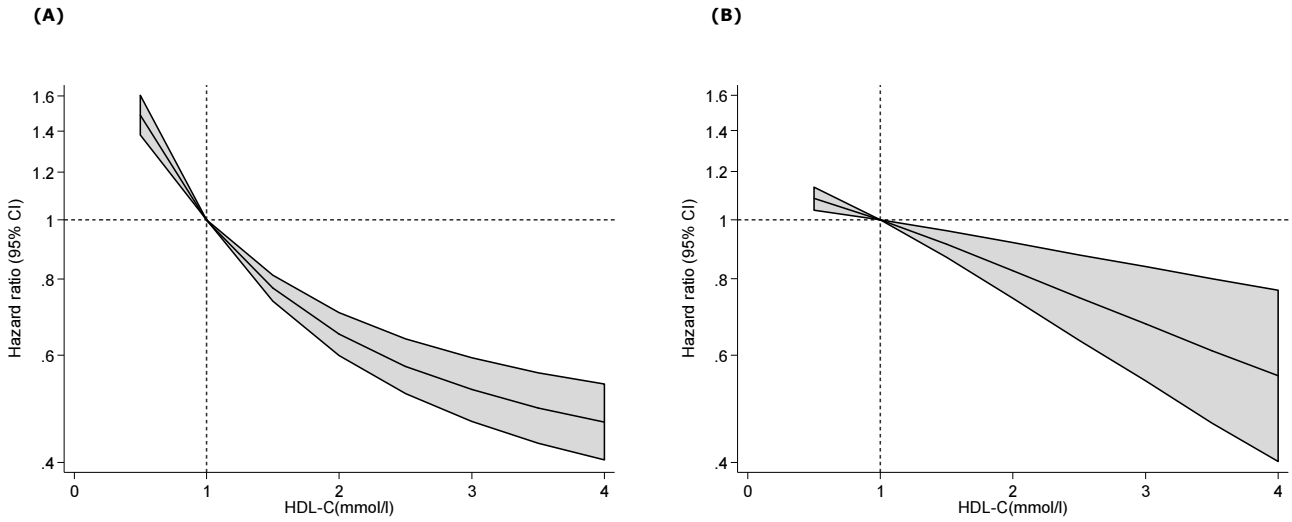
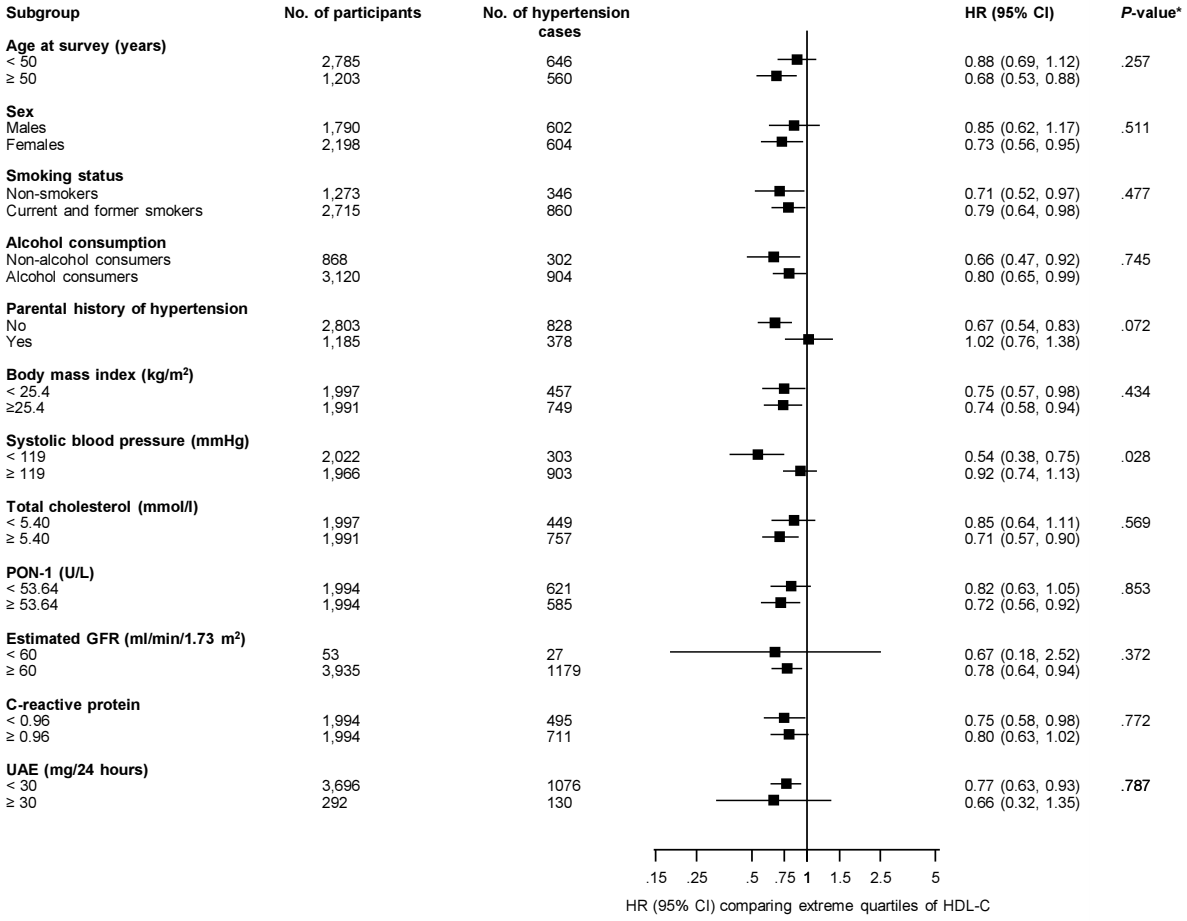
Figure 1. Hazard ratios for incident hypertension using multivariate-adjusted fractional polynomials



A, Hazard ratios were adjusted for age and sex; **B**, adjustment in A plus smoking status, history of type 2 diabetes, systolic blood pressure, total cholesterol, body mass index, parental history of hypertension, alcohol consumption, and estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); HDL-C, high-density lipoprotein cholesterol

Figure 2. Hazard ratios of HDL-C and incident hypertension risk by several participant level characteristics



Hazard ratios were adjusted for age, sex, smoking status, history of type 2 diabetes, systolic blood pressure, total cholesterol, body mass index, parental history of hypertension, alcohol consumption, and estimated glomerular filtration rate (GFR) (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); CI, confidence interval (bars); HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; hs, high-sensitivity; PON-1, paraoxonase-1; UAE, urine albumin excretion; *, *p* value for interaction; cut-offs used for body mass index, systolic blood pressure, total cholesterol, PON-1, and hsC-reactive protein are median values.

Table 1. Baseline Participant Characteristics Overall and According to the Development of Incident Hypertension

	Overall (N=3,988) Mean (SD) or median (IQR) or n (%)	**Without incident hypertension (N=2,782) Mean (SD) median (IQR) or n (%)**	**With incident hypertension (N=1,206) Mean (SD) or median (IQR) or n (%)**	***p* value[a]**
Serum paraoxonase-1 (U/L)	56.4 (18.0)	56.6 (17.9)	55.9 (18.1)	0.254
Questionnaire				
Males	1,790 (44.9)	1,188 (42.7)	602 (49.9)	< 0.001
Age at survey (years)	45 (11)	43 (10)	50 (10)	< 0.0001
History of type 2 diabetes	19 (0.5)	9 (0.3)	10 (0.8)	0.033
Smoking				
Current	1,364 (34.2)	941 (33.8)	423 (35.1)	
Former	1,351 (33.9)	914 (32.9)	437 (36.2)	0.012
Never	1,273 (31.9)	927 (33.3)	346 (28.7)	
Alcohol consumers	3,121 (78.2)	2,216 (79.7)	904 (75.0)	0.001
Parental history of hypertension	1,185 (29.7)	807 (29.0)	378 (31.3)	0.138
Physical measurements				
BMI (kg/m^2)	25 (4)	25 (3)	26 (4)	< 0.0001
WHR	0.86 (0.09)	0.85 (0.08)	0.88 (0.09)	< 0.0001
SBP (mmHg)	119 (11)	116 (10)	125 (10)	< 0.0001
DBP (mmHg)	70 (7)	68 (7)	74 (7)	< 0.0001
Lipid, metabolic, inflammatory, and renal markers				
Total cholesterol (mmol/l)	5.48 (1.11)	5.35 (1.07)	5.77 (1.14)	< 0.0001
HDL-cholesterol (mmol/l)	1.38 (0.40)	1.41 (0.40)	1.32 (0.41)	< 0.0001
Non-HDL-cholesterol (mmol/l)	4.10 (1.19)	3.94 (1.14)	4.45 (1.24)	< 0.0001
Apo A-1 (g/l)	1.40 (0.30)	1.40 (0.30)	1.39 (0.30)	0.138
Apo B (g/l)	0.98 (0.30)	0.95 (0.29)	1.07 (0.30)	< 0.0001
Glucose (mmol/l)	4.63 (0.87)	4.55 (0.71)	4.82 (1.13)	< 0.0001
Fasting insulin (units/ml)	7.1 (5.1-10.2)	6.8 (4.9-9.6)	7.8 (5.5-11.6)	< 0.0001
HOMA-IR	1.43 (0.99-2.12)	1.36 (0.96-1.94)	1.65 (1.10-2.50)	< 0.0001
hsCRP (mg/l)	0.95 (0.43-2.30)	0.86 (0.38-2.00)	1.29 (0.58-2.87)	< 0.0001
eGFR (ml/min/1.73 m^2)	92.3 (14.0)	93.7 (13.5)	88.9 (14.3)	< 0.0001
UAE (mg/24 hours)	8.04 (5.86-12.45)	7.62 (5.72-11.48)	9.18 (6.38-15.55)	< 0.0001

BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; SBP, systolic blood pressure; UAE, urinary albumin excretion; WHR, waist-to-hip ratio

[a], employed a two-sample *t*-tests for a difference in means for continuous variables and a chi square test for categorical variables

Table 2. Cross-sectional correlates of paraoxonase-1

	Partial correlation r (95% CI)[a]	Absolute difference (95% CI) in PON-1 activity per 1 SD higher or compared to reference category of correlate[b]
Serum paraoxonase-1 (U/L)	-	-
Sex		
Female	-	Ref
Male	-	-3.07% (-4.18, -1.95)***
Questionnaire		
Age at survey (years)	-0.05 (-0.08, -0.02)**	-0.91% (-1.46, -0.35)*
History of type 2 diabetes		
No	-	Ref
Yes	-	-6.02% (-14.11, 2.07)
Smoking status		
Non-smokers	-	Ref
Current and former smokers	-	-0.41% (-1.61, 0.78)
Alcohol consumption		
Non-consumers	-	Ref
Current consumers	-	3.21% (1.85, 4.57)***
Parental history of hypertension		
No	-	Ref
Yes	-	-0.51% (-1.74, 0.71)
Physical measurements		
BMI (kg/m^2)	-0.02 (-0.05, 0.01)	-0.37% (-0.94, 0.19)
WHR	-0.04 (-0.07, -0.01)***	-0.88% (-1.62, -0.13)*
SBP (mmHg)	0.05 (0.02, 0.08)	0.98% (0.37, 1.59)*
DBP (mmHg)	0.04 (0.00, 0.07)	0.71% (0.10, 1.31)*
Lipid, metabolic, inflammatory, and renal markers		
Total cholesterol (mmol/l)	0.10 (0.07, 0.13)***	1.95% (1.6, 2.53)***
HDL-cholesterol	0.16 (0.13, 0.19)***	3.28 (2.67, 3.89)***
Non-HDL-cholesterol (mmol/l)	0.04 (0.01, 0.07)	0.81% (0.21, 1.40)*
Apo A-1 (g/l)	0.16 (0.13, 0.19)***	3.05% (2.47, 3.63)***
Apo B (g/l)	0.02 (-0.01, 0.05)	0.45 (-0.13, 1.04)
Glucose (mmol/l)	-0.05 (-0.08, -0.02)***	-0.98% (-1.56, -0.41)**
Log_e Fasting insulin (units/ml)	0.03 (0.00, 0.06)	0.57% (0.01, 1.12)*
Log_e HOMA-IR	0.02 (-0.02, 0.05)	0.28% (-0.29, 0.84)
Log_e hsCRP (mg/l)	-0.03 (-0.06, -0.00)*	-0.60% (-1.16, -0.04)*
eGFR (ml/min/1.73 m^2)	0.01 (-0.02, 0.04)	0.22% (-0.46, 0.90)
Log_e UAE (mg/24 hours)	0.02 (-0.01, 0.05)	0.30% (-0.26, 0.86)

BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; Ref, reference; SD, standard deviation; SBP, systolic blood pressure; UAE, urinary albumin excretion; WHR, waist-to-hip ratio

Asterisks indicate the level of statistical significance: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; [a], Pearson correlation coefficients between paraoxonase-1 and the row variables; [b], Absolute change in paraoxonase-1 values per 1 SD increase in the row variable (or for categorical variables, the absolute difference in mean paraoxonase-1 values for the category versus the reference) adjusted for age and sex;

Table 3. Association of serum paraoxonase-1 activity with incident hypertension

PON-1 activity (U/L)	Events/ Total	Model 1		Model 2		Model 3		Model 4	
		HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Per 1 SD increase	1,206 / 3,988	1.01 (0.96 to 1.07)	0.656	0.99 (0.93 to 1.05)	0.781	0.99 (0.93 to 1.05)	0.764	1.00 (0.94 to 1.06)	0.936
Q1 (0.84-43.95)	316 / 997	ref		ref		ref		ref	
Q2 (43.95-53.63)	305 / 997	0.99 (0.84 to 1.16)	0.875	0.89 (0.76 to 1.04)	0.143	0.89 (0.76 to 1.04)	0.155	0.90 (0.77 to 1.06)	0.206
Q3 (53.64-65.85)	284 / 997	0.96 (0.82 to 1.13)	0.658	0.88 (0.75 to 1.03)	0.116	0.88 (0.75 to 1.04)	0.129	0.91 (0.77 to 1.07)	0.233
Q4 (65.85-148.68)	301 / 997	1.07 (0.91 to 1.25)	0.433	0.98 (0.83 to 1.15)	0.788	0.98 (0.83 to 1.15)	0.806	1.01 (0.86 to 1.19)	0.919

PON-1, paraoxonase-1; Q, quartile; SD, standard deviation

Model 1: Age and sex

Model 2: Model 1 plus smoking status, history of type 2 diabetes, systolic blood pressure, total cholesterol, body mass index, parental history of hypertension, alcohol consumption, and estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation)

Model 3: Model 2 plus $\log_e$ urinary albumin excretion and $\log_e$ homeostasis model assessment of insulin resistance, and $\log_e$ high-sensitivity C-reactive protein

Model 4: Model 3 plus high-density lipoprotein cholesterol

Table 4. Association of serum high-density lipoprotein cholesterol with incident hypertension

Serum HDL-cholesterol (mmol/l)	Events/ Total	Model 1		Model 2		Model 3		Model 4	
		HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Q1 (0.36-1.08)	396 / 1,010	ref		ref		ref		ref	
Q2 (1.09-1.32)	285 / 984	0.69 (0.59 to 0.80)	< 0.001	0.76 (0.65 to 0.89)	0.001	0.76 (0.65 to 0.89)	0.001	0.76 (0.65 to 0.89)	0.001
Q3 (1.33-1.63)	266 / 1,007	0.60 (0.51 to 0.71)	< 0.001	0.74 (0.62 to 0.87)	< 0.001	0.76 (0.64 to 0.90)	0.002	0.76 (0.64 to 0.90)	0.002
Q4 (1.64-3.42)	259 / 987	0.62 (0.52 to 0.74)	< 0.001	0.76 (0.63 to 0.92)	0.005	0.80 (0.66 to 0.98)	0.028	0.80 (0.66 to 0.98)	0.030

HDL, high-density lipoprotein; Q, quartile

Model 1: Age and sex

Model 2: Model 1 plus smoking status, history of type 2 diabetes, systolic blood pressure, total cholesterol, body mass index, parental history of hypertension, alcohol consumption, and estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation)

Model 3: Model 2 plus $\log_e$ urinary albumin excretion and $\log_e$ homeostasis model assessment of insulin resistance, and $\log_e$ high-sensitivity C-reactive protein

Model 4: Model 3 plus paraoxonase-1